Proposed synopsis on

"EVALUATION OF AN ETHNOMEDICINAL PLANT EXTRACTS AND BIOACTIVITY GUIDED ISOLATION OF PHYTOCONSTITUENT FOR ANTI-ASTHMATIC POTENTIAL''

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ABSTRACT

Asthma is a complex, chronic inflammatory disease of the lower airways affecting people of all ages. Approximately 300 million individuals are currently suffering from asthma worldwide and 10 per cent of it *i.e.* 30 million in India. The prevalence of asthma is estimated to range from 3 to 38 per cent in children and from 2 to 12 per cent in adults and it has been estimated that a further 100 million will be affected by 2025. Since the ancient times, plants have been exemplary sources of medicine.

Current synthetic drugs belonging to different classes such as α_2 agonists (Ephedrine), corticosteroids (Hydrocortisone, Prednisolone), mast cell stabilizers (Sodium chromoglycate, Kitotifen), methylxanthines (Theophylline, Aminophylline), leucotriene antagonists (Montelukast, Zafirlukast) are widely used in the treatment of asthma therapy. All these drugs in more or less manner show adverse effects which raises question of safety for long term use. The most common side effects shown by these drugs are immune suppression, cardiac abnormalities, central nervous system depression and hyperglycemia. In addition, muscle tremor and hypokalemia are the major adverse effects of β_2 agonists. Systemic corticosteroids have common side effects such as disturbance of adrenal function and immune suppression. Therefore, there is a dreadful demand to identify an effective and safe remedy for the treatment of bronchial asthma. The traditional systems of medicine and the accessibility of a large array of medicinal plants in the universe have significantly facilitated the researchers to find solution of said problem. Screening of traditionally acclaimed medicinal plants to develop novel medicine for asthma is a need of an hour.

Research conducted in the last few decades on the ethno pharmacologically claimed plants for asthma have shown anti-histaminic and anti-allergic activity of studied medicinal plants. One of the basic objectives in medicinal plant research and development is identification, isolation and characterization of bioactive component present in the extract. The phytochemical research based on ethnopharmacological profile is considered as one of the fundamental approach towards discovery of novel bioactive chemical entities with potential as drug leads. With this view present study is aimed for **"Evaluation of an ethnomedicinal plant extracts and bioactivity guided isolation of phytoconstituent for anti-asthmatic potential''**

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells. Asthmatic patients experience intermittent episodes of wheezing, shortness of breath, chest tightness and cough, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyper-responsiveness to a variety of stimuli. As of 2009, 300 million people worldwide were affected by asthma leading to approximately 250000 deaths per year and 10 per cent of it *i.e.* 30 million in India.

Most patients with asthma achieve good disease control with principal use of inhaled corticosteroids and long-acting β_2 -adrenoceptor agonists that are the mainstay of asthma therapy. These therapies, however, unfortunately fail to afford a significant proportion of patients with good control of their symptoms or prevent severe exacerbations. There are a number of possible reasons for this limitation in the efficacy of traditional therapies, including 1) a failure of traditional guidelines to reflect patients' own priorities of asthma control; 2) inappropriate timing of the introduction of treatments or the use of inadequate doses; 3) a poor understanding of different asthma subgroups, which due to their distinct pathophysiology have different pharmacological responses; 4) limitations in the ability of patients to adhere to prescribed regimens; and 5) a proportion of patients genuinely having severe disease who are resistant to treatment (difficult to treat asthma).

The current 'gold standard' of asthma therapy is a combination inhaler containing a long acting β_2-agonist with a corticosteroid an improved form of adrenal gland extract. Cromoglycate, derived from a plant product and theophylline, a dietary methyl xanthine, have also been extensively used in the therapy of asthma, but we still do not understand their molecular mechanisms. Pharmacology has played an important role in improving natural products to make effective long lasting and safe asthma therapies, but has so far been challenged to produce new classes of antiasthma therapy. The only novel class of antiasthma therapy introduced in the last 30 years are leukotriene antagonists, which are less effective than existing treatments.

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Plant biodiversity forms the most common source of medicines and therefore there has been a shift towards the traditional herbal medicines either directly or indirectly. The medicinal properties offered by the plants are due to bioactive phytoconstituents such as poly phenols (flavonoids and tannins), terpenoid, alkaloids, steroids, saponins etc. These secondary metabolites and their derivatives exhibited significant biological and pharmacological properties.

Medicinal plants used for treatment of asthma is anticipated to have shown interesting findings in various target specific biological activities such as bronchodilation, mast cell stabilization, anti-anaphylactic, anti-inflammatory, anti-spasmodic, anti-allergic, immunomodulatory and inhibition of mediators such as leukotrienes, lipoxygenase, cyclooxygenase, platelet activating, phosphodiesterase and cytokine, in the treatment of asthma.

REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT

Mali R G & Dhake A S (2011) in their review attempted to classify the pharmacological and preclinical findings based on the possible action mechanism of 157 reported medicinal plants. Paper also signifies the need of pertinent research finding various potential of ethno botanically claimed herbs acting at particular sites of the pathophysiological cascade of asthma for prophylaxis as well as treatment of asthma.

Sasmal S *et al*, (2012) studied the pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of acetone extract the seeds of S*araca asoca* Roxb.

Apiamu A *et al*, (2013) studied the effect of aqueous leaves extract of *Euphorbia heterophylla*. Phytochemical analysis of this plant confirms the presence of various phytochemicals like saponins, steroids, terpenoids, alkaloids, carbohydrates, tannins in its extract. However this plant has not been evaluated for anti-asthmatic activity.

Rimjhim S et al, (2014) has performed the qualitative phytochemical analysis of leaves of *Clerodendrum infortunatum,* which confirms the presence of various phytochemicals like sterols, terpenoids, alkaloids, carbohydrates and tannins in its methanolic extract, but have not been isolated and evaluated for anti-asthmatic activity.

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Oghale O U & Idu M D (2016) have studied the phytochemistry, anti-asthmatic and antioxidant activities of the aqueous leaf extract of *Anchomanes difformis* (Blume) Engl. and verified its claims of use in folk medicine. Author's evaluated effect on tracheal fluid viscosity, tracheal fluid volume, histamine induced broncho constriction in guinea pigs of standardised extracts.

Suchitra et al, (2017) studied the comparative phytochemical and antioxidant properties of *Costus pictus* and *C. speciosus*, showed the appreciable amount of bioactive compound and also compare the two species by their phytochemical nature, qualitative HPTLC fingerprinting profile and their chemical nature screening for antioxidant activity.

Literature revealed that there are number of medicinal plants which have been claimed in traditional system of medicine for its effectiveness in respiratory disorders like asthma but lacks systematic studies of these plants. In addition, certain plants have been evaluated for preliminary anti-asthmatic activity, but extensive screening including bioactive guided isolation; characterization and expression of plausible action mechanism/ pharmacology have not been done. Therefore it will be attempted to design the present study with an intention to explore anti-asthmatic potential of ethno botanically claimed medicinal plants with best possible evaluation models available.

Accordingly, for present study following ethnopharmacologically claimed medicinal plants are shortlisted so as to select any one or two medicinal plant. Further literature will be carried out, if needed to select any other medicinal or ethnopharmacologically claimed plant/s for the said pharmacological study. With this view present study is aimed for **"Evaluation of an ethnomedicinal plant extracts and bioactivity guided isolation of phytoconstituent for anti-asthmatic potential''**

1. *Saraka Asoca* (Caesalpinaceae)
2. *Costus Speciosus* (Zingiberaceae)
3. *Abelmoschus ficulnesus* (Malvaceae)
4. *Euphorbia heterophylla* (Euphorbiaceae)
5. *Calotropis Pocera* (Asclepiadaceae)
6. *Calotropis gigantea* (Asclepiadaceae)
7. *Ferula Narthes* (Umbelliferae)
8. *Clendendrum Infortunatum* (Verbenaceae)

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OBJECTIVES OF THE RESEARCH

In this modern era for primary health care, most of the world population still uses herbal medicine because of its better cultural acceptability, better compatibility with the human body and lesser side effects. Present study is aimed for "Evaluation of an ethnopharmacologically selected medicinal plant extract and bioactivity guided isolation of phyto constituent for anti-asthmatic potential''

The said aim will be implemented with following objectives

a) Phytochemical screening and investigation of selected medicinal plant extracts.
b) Standardization of plant extracts for contents of phytoconstituents by means of suitable spectroscopic and chromatography methods.
c) Toxicity study of extracts to obtain safety data
d) Preliminary screening for antioxidant and anti-inflammatory (*in vitro* & *in vivo* methods) potential of plant extracts.
e) Pharmacological evaluation of plant extracts for its anti-asthmatic activity (i*n vitro* & *in vivo* methods).
f) Bioactivity guided fractionation of extracts/fraction.
g) Isolation, characterization and pharmacological evaluation of bioactive phytoconstituent/s from the plant extract/fraction.

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METHODOLOGY TO BE ADDOPTED

1. **Collection of plant material:**

   The whole plant or its parts will be collected from the known sources. The collected plant specimen will be identified and authenticated from official resources.

2. **Processing of plant material:**

   Collected plant material will be subjected to processing such as drying, grinding, sieving etc. to make it suitable for extraction.

3. **Extraction of plant material with various solvents:**

   Coarse powder form of plant material will be subjected to successive extraction. The solvent & extraction method will be optimized according to nature of phytoconstituents.

4. **Phytochemical screening and standardization of plants extracts:**

   All the extracts will be subjected for phytochemical screening (test for different phytoconstituents and TLC fingerprinting). Standardization of extracts will be carried for the estimation a total polyphenols, alkaloids, carbohydrates, proteins, lipids, saponin, steroids content by means of suitable spectroscopic and chromatography methods.

5. **Pharmacological screening of plant extracts:**

   Plant material extracts will be evaluated for

   i. Determination of acute oral & repeated dose toxicity:

   Acute oral & repeated dose toxicity studies will be carried out for extracts as per suitable OECD guideline.

   ii. Pharmacological screening for antioxidant activity:

   Plant extracts will be screened by methods like % DPPH, % RRI DPPH, ABTS, metal chelation and reducing power etc.

   iii. Pharmacological screening for anti-inflammatory activity:

   Plant extracts will be screened by methods like carrageenan induced rat paw edema, egg albumin model). Anti-inflammatory evaluation will assist to understand possible role of plant extract/s in asthma like respiratory disorders.

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iv. Pharmacological screening for anti-asthmatic activity:

After preliminary phytochemical, antioxidant & anti-inflammatory evaluation of plant extracts/fractions, the most effective plant extracts/ fractions will be subjected to bronchodilatory and bronchoprotective effect using suitable *in-vitro* and or *in-vivo* preclinical model.

6. **Bioactivity guided isolation of phytoconstituents from fractions or extracts:**

   The most bioactive and potent extract/fraction resulted from afore mentioned screening will be subjected to bioactivity guided fractionation, separation and isolation, which will be carried out by using pertinent separation techniques.

7. **Characterization and pharmacological screening of bioactive phytoconstituent/s:**

   Separated, isolated & purified phytoconstituent/s will be characterized using suitable spectroscopic techniques. The isolated phytoconstituent/s will be screened by selected pharmacological evaluation methods.

IMPORTANCE OF STUDY/ SOCIETY APPLICATION

Herbal medicine, as a major part of traditional medicine, has been used in medical practice since ancient times and is a common element of ayurvedic, homeopathic and naturopathic medicine.

World Health Organization (WHO) estimates that 65% of the world's population uses traditional medicines as their primary form of health care and about 85% of traditional medicines involve the use of herbal preparations by native cultures. The worldwide market of herbal drugs at present is approximately $ 600 billion.

India has a rich cultural heritage of traditional medicines. The crude drugs being always available easily in abundance, comparatively cheaper, with negligible side effects and have frequently been prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books. Among several respiratory diseases affecting human, bronchial asthma is the most common disabling syndrome with a worldwide incidence of 155 millions. It is a disease that does not have the boundaries of age, race and gender. The availability of effective medications is not withstanding with present challenges and the prevalence of asthma is continuously increasing with time Moreover the side effects.

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A regular and widespread use of herbs throughout the world has increased serious concerns over their quality, safety and efficacy. Screening of traditionally acclaimed medicinal plants to develop novel medicine for asthma by utilizing cutting edge evaluation methodologies and to present scientifically validated data is a need of an hour.

Followings are the number of herbs that are ethno pharmacologically claimed for the management of respiratory disorders (including asthma) like *Picrorrhiza kurroa, Adhatoda vasica, Albizzia lebbek, Solanum xanthocarpum, Tylophora indica, Cedrus deodara, Boswellia serrata, Phyllanthus urinaria, Aquilaria agallocha, Calotropis procera, Elettaria cardamomum, Ocimum sanctum, Piper longum, Tamarindus indica, Hemidesmus indicus, Balanites roxburghii, Centella asiatica* etc. Existing therapies for the treatment of the respiratory disorders are not complete and satisfactory due to major side effects at higher doses and prolonged treatment. Present scenario demands a need of development of newer treatment with safe approach/ lesser adverse effects. Therefore, there is a dire need to identify effective and safe remedies to treat bronchial asthma. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care. Assessing the current status of health care system in adequacies of synthetic drugs is likely to be more glaring in the coming years.

Selected medicinal plant/its parts are less explored in terms of preclinical and/ clinical aspects, however all these are ethno pharmacologically claimed to possess important phytoconstituents of wide range which will be useful in respiratory disorders like asthma. Such plats extracts, fractionated constituents can be separated, and evaluated using modern tools for analytical, pharmacological and toxicological profile. Present project may help to explore and establish scientifically proved data for novel constituents for the mentioned pathologies which could be more effective as well as associated with lesser side effect which will be beneficial for the society.

With this regards present project is undertaken with objectives to identify, isolate and characterize bioactive component present in the selected some medicinal plant extract and to evaluate its antiasthmatic activity.

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PROPOSED WORK PLAN

1. **Collection, identification and authentication of selected medicinal plant material.**
2. **Processing of crude drug for extraction**
3. **Extraction of plant material:**

a) Selection of solvent for extraction

b) Selection and optimization of extraction method

4. **Phytochemical screening and standardization of plants extracts:**

   Phytochemical screening of both qualitative and quantitative analysis for TLC fingerprinting of extracts and standardization of extracts by estimating total carbohydrates, proteins, lipids, polyphenols, flavonoid, saponin, alkaloids, steroids content etc

5. **Pharmacological screening of plant extract(s)/fraction(s) for:**

   i) Acute oral & repeated dose toxicity

   ii) Antioxidant activity

   iii) Anti-inflammatory activity

   iv) Anti-asthmatic activity

6. **Bioactivity guided isolation of phytoconstituents from fractions or extracts:**

   The most bioactive and potent extract(s)/ fraction(s) resulted from afore mentioned screening will be subjected to bioactivity guided fractionation, separation and isolation, which will be carried out by using pertinent separation techniques.

7. **Characterization and pharmacological screening of bioactive phytoconstituent/s:**

   Separated, isolated & purified phytoconstituent/s from bioactive extract(s)/ fraction(s) will be characterized using suitable spectroscopic techniques. The isolated phytoconstituent/s will be screened by selected pharmacological evaluation methods.

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YEAR WISE PLAN OF WORK AND TARGET TO ACHIEVE

Present study is designed in such a way that it will come to a conclusive outcome within 03 years from the date of allocation. The year wise plan includes**,**

YEAR 01

- Literature survey;
- Collection, identification and authentication of plant material;
- Processing of authenticated plant material such as drying, segregation, grinding, sieving etc. to make it suitable for extraction;
- Preliminary screening, physical evaluation of powdered drug.

YEAR 02

- Extraction of the plant material by selected and optimized method of extraction;
- Assessment for presence of important phytoconstituents in extracts/ fractions by different qualitative and quantitative methods of evaluation as well as standardization;
- *In vitro* pharmacological screening of different extracts/fractions for anti-oxidant, anti-inflammatory and anti -asthmatic activity.

YEAR 03

- *In vivo* pharmacological screening of different extracts/fractions, for anti-oxidant, anti-inflammatory and anti-asthmatic activity using suitable screening methods;
- Bioactivity guided fractionation, separation and isolation of phytoconstituents from fractions or extracts by using pertinent separation techniques;
- Characterization of phytoconstituents using suitable spectroscopic techniques;
- Pharmacological screening of bio active phytoconstituents using selected pharmacological evaluation methods;
- Compilation and interpretation of data;
- Communication of research and/or review articles and thesis writing.

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